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# Prognostic significance of inflammatory factors expression by stroma from breast carcinomas

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## ABSTRACT

The aim of this work was to evaluate the expression and clinical relevance of some cytokines in breast carcinomas. An immunohistochemical study using tissue arrays and specific antibodies against interleukin-1β (IL-1β), IL-6, IL-10, IL-17, interferon β (IFNβ) and nuclear factor kappa B (NFκB), was performed in 108 breast carcinomas. Most studied cytokines were mainly expressed by cancer cells, but also by stromal cells as cancer-associated fibroblasts (CAFs) or mononuclear inflammatory cells (MICs). Global expression (score) of IL-1β and IL-17 was positively associated with histological grade; HER-2-positive tumors showed a higher global expression of IFNβ but a lower global expression of NFκB; and node-negative tumors showed a higher global expression of IL-6. High score of IL-6 was significantly associated with both longer relapse free-survival (RFS) and overall survival (OS). Moreover, the expression of IL-1β by each stromal cells (CAFs and MICs) was significantly associated with both longer RFS and OS; whereas the expression of IL-10 by these cells was significantly associated with both shorter RFS and OS. However, the combination of IL-1β, IL-6 and IL-10 expression by MICs reached an important association with prognosis and improved our previously reported prognostic signification based on the MMP-11 status by MICs. The combination of IL-1β, IL-6 and IL-10 expression by MICs was significant and independently associated with distant relapse-free survival in a multivariate analysis. Therefore, the combination of the expression of IL-1β, IL-6 and IL-10 may serve as promising biomarkers of MICs with prognostic significance, contributing to a better characterization of breast carcinomas microenvironment.

**Summary:** The combination of the expression of IL-1b, IL-6 and IL-10 may serve as promising biomarkers of MICs with prognostic significance, contributing to a better characterization of breast carcinomas microenvironment.

**Keywords:** IL-1β, IL-6, IL-10, IL-17, IFNβ, NFκB, metastasis, inflammation, breast cancer.

## INTRODUCTION

Metastasis is the major cause of mortality in patients with breast cancer. Unfortunately, nowadays it is difficult to predict metastatic relapse or prevent it therapeutically. Gene expression analysis of tumor samples has been a valuable tool to characterize tumors and could have prognostic and predictive value. However, breast cancer is a complex and heterogeneous disease, and its prognosis may depend on tumor and host characteristics (1). Over the last years, growing evidences indicate that breast cancer microenvironment plays a crucial role in tumorigenesis, from initiation to progression. Tumor microenvironment is a complex combination of different cell types and molecules, and is a key contributor to malignant progression (2). Tumor microenvironment includes two well-studied cellular component of tumor stroma: cancer-associated fibroblasts (CAFs) and mononuclear inflammatory cells (MICs) (including macrophages, T and B lymphocytes).

The immune system plays a complex role in tumorigenesis (3). Although tumor-infiltrating leukocytes have been historically considered to be manifestations of an intrinsic defense mechanism against developing tumors (4), the presence of leukocytes in tumors was subsequently interpreted as an aborted attempt of the immune system to reject the tumor. Nowadays, increasing evidence indicates that leukocyte infiltration can promote tumor phenotypes, such as angiogenesis, growth and invasion (5-6). Some inflammatory mediators, such as cytokines, are important factorsof tumor microenvironment.

Previously, we had found that breast carcinomas containing MICs or CAFs with a high expression profile of matrix metalloproteases (MMPs) and their tissue inhibitors (TIMPs) had a higher rate of distant metastasis development compared with tumors with a low expression

profile (7-10). In tumors with a high expression profile of MMPs/TIMPs, MMP-11 (also known as stromelysin 3) was the most frequently expressed factor by MICs. These are relevant findings because it is known that MMPs and TIMPs exert powerful influences on local microenvironment during tumorigenesis and tumor progression.

Considering that the expression of MMP-11 may constitute a useful biological marker for pro-metastatic MICs, in previous studies, we investigated its relationship with the expression of 65 molecular factors associated with inflammation and tumor progression in a population of breast cancer patients stratified in two groups according to MMP-11 expression by intratumoral MICs(11-12). Among all of these analyzed factors, IL-1β, IL-6, IL-17, IFNβ and NFκB, were expressed at high levels (analyzed by real time PCR) in tumors with MMP-11 positive MICs. Consequently, in the present study we analyzed, in breast carcinomas, the protein expression levels (score) of these cytokines (IL-1β, IL-6, IL-17, IFNβ, NFκB) and IL-10, and also their expression by cancer cells, CAFs and MICs, using tissue arrays (TA) and immunohistochemical techniques. Our results point to the relevance of the co-expression of IL-1β, IL-6 and IL-10 in MICs, as a biological marker of prognostic importance that contribute to a better characterization of tumor stroma in breast cancer.

## MATERIAL AND METHODS

### Patient selection

This study comprised 108 women with a histologically confirmed diagnosis of early invasive breast cancer of ductal-type treated between 1990 and 2001. Some of them were previously included in our previous studies about the expression of MMPs and TIMPs in breast cancer (7-10). We selected women with the following inclusion criteria: invasive ductal carcinoma, T1 or T2 tumor size, at least 6 histopathologically-assessed axillary lymph nodes, and a minimum of 10 years of follow-up in those women without tumor recurrence. The exclusion criteria were the following: metastatic disease at presentation, prior history of any type of malignant tumor, bilateral breast cancer at presentation, having received any type of neoadjuvant therapy, development of loco-regional recurrence during the follow-up period, development of a second primary cancer, and absence of sufficient tissue in the paraffin blocks used for manufacturing the tissue arrays. We randomly selected a sample size of 108 patients, in accordance to 4 different groups of similar size and stratified with regard to nodal status and to the development of metastatic disease, which were key variables in our study. Thus, we include a sufficient number of both node-negative and node-positive patients to comprise each sub-group among patients without and with disease recurrence for securing the statistical power of the survival analysis. Patients' characteristics included in the main groups, with or without distant metastases (recurrence), are listed in Supplementary Table 1. Patients underwent either modified radical mastectomy or wide resection with axillary lymphadenectomy. Data about the criteria for systemic adjuvant therapy or postoperative radiotherapy of the patients were described elsewhere (7). The median follow-up period in patients without metastasis was of 138 months, and 26 months in patients with metastatic

disease. The study adhered to national regulations and was approved by our Institution's Ethics and Investigation Committee.

**Tissue arrays (TAs) and immunohistochemistry**

Routinely fixed (overnight in 10% buffered formalin), paraffin embedded tumor samples stored in our pathology laboratory archives were used in this study. TAs blocks were obtained by punching a tissue cylinder (core) with a diameter of 1.5 mm into a histologically representative area of each 'donor' tumor block, which was then inserted into an empty 'recipient' tissue array paraffin block using a manual tissue arrayer (Beecher Instruments) as described elsewhere (7). A total of 2 cores were employed for each case, corresponding to tumor center area. This method, with 2 cores (double redundancy) of each tumor area has been shown to correlate well with conventional immunohistochemical staining (7). Immunohistochemistry was carried out using a TechMate TM50 autostainer (Dako). Antibodies used were listed in Supplementary Table 2. The negative control used was DakoCytomation mouse serum diluted to the same concentration as the primary antibody. As positive controls, we used breast tumour samples in which the presence of the evaluated factors show the lowest or the highest expression by PCR, as described in a prior report(11). Endogenous peroxidase activity was blocked by incubating the slides in peroxidase-blocking solution (Dako) for 5 min. The EnVision Detection Kit (Dako) was used as the staining detection system. Sections were counterstained with hematoxylin, dehydrated with ethanol and permanently coverslipped.

**Double staining**

Double-immunostaining methods were performed with the BenchMark ULTRA Staining System (Ventana-Roche, Rotkreuz, Switzerland). Paraffin-embedded tissue sections were pre-treated at 95ºC for 52 min in buffer pH 8, and incubated with the following primary antibodies: IL-1β (1:100, 84 min) and the CD45Ro marker (ready-to-use (Roche), 36 min) specific for MICs, or Cytokeratin AE1/AE3 (ready-to-use (Roche), 24 min) specific for cancer cells and the α-SMA marker (ready-to-use (Roche), 40min) specific for fibroblasts.

**Immunostaining evaluation**

For each factor, we have determined the global expression (score) and the expression by different cell types.

**Global expression – score determination**

All the cases were semiquantified for each protein-stained area. An image analysis system with the Olympus BX51 microscope and soft analysis (analySIS, Soft imaging system, Münster, Germany) were used as follows employed as follows: tumor sections were stained with antibodies according to the method explained above and counterstained with hematoxilin. There are different optical thresholds for both stains. Each core was scanned with the x40 objective lens in two fields per core. The computer program selects and traces a line around antibody-stained areas (higher optical threshold: red spots), with the remaining non-stained areas standing out as a blue background (supplementary Figure 1). Each field has an area ratio of stained *vs*. non-stained areas. A final area ratio was obtained after averaging two fields.

In addition, the immunostaining intensity was evaluated using a numeric score ranging from 0 to 3, reflecting the intensity as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, intense staining (supplementary Figure 2).

Using an Excel spreadsheet, the score was obtained by multiplying the intensity score (I) by the area ratio (R) (Score= I x R). This score was then averaged with the number of cores that were carried out for each patient (n= 2 cores per patient).

Staining for estrogen receptors (ERs) and progesterone receptors (PgRs) was scored according to the method described by Allred *et al*. (13) and HER-2 staining according to the criteria used for the Herceptest. In addition, we established the following subtypes: no basal-like tumors and basal-like (ER-, PgR-, HER2-) (14).

**Determination of cell type expression**

Two certified pathologists (LOG and FD), blinded to the clinical outcome of the patients, performed the histological examination. In each case, immunoreactivity was classified into 2 categories depending upon the percentage of cells stained per field (negative: 0–10% positive cells; positive: >10% positive cells) in each cell type (cancer cells, CAFs and MICs), independently of the staining intensity. Each evaluated field (x40 objective lens) contained at least 10 CAFs and 10 MICs, as it was established in a previous study(8). We distinguished stromal cells from cancer cells on the basis of cell size (the latter cells are larger in size). Stromal cell subsets were distinguished primarily by morphology (CAFs are spindle shaped cells, whereas MICs are small round cells). Additionally, whereas cancer cells are arranged forming either acinar or trabecullar patterns, stromal cells are scattered throughout the tissue. To confirm that the morphology described is in accordance to the cell type, we used specific markers to distinguish cancer cells (Cytokeratin AE1/AE3), cancer-associated fibroblasts (α-SMA) and mononuclear inflammatory cells (CD45Ro).

**Data analysis and statistical methods**

Differences in percentages were calculated with the chi-square test. Immunostaining score of each protein were expressed as median (range). Comparison of immunostaining values

between groups was made with the Mann–Whitney or Kruskal–Wallis tests. For metastasis-free survival analysis, we used the Cox univariate method. Cox's regression model was used to examine interactions of different prognostic factors in a multivariate analysis. $p \leq 0.05$ was considered as significant. The SPAW Statistics 18.0 software was used for all calculations (SPSS Inc.).

## RESULTS

More than 1,200 determinations were performed on TAs from 108 patients with breast carcinoma.

Figure 1A shows some examples of TA with immunostaining for each protein evaluated. Most studied cytokines were mainly expressed by cancer cells in breast carcinomas. However, these proteins were also expressed by CAFs and by MICs in a significant percentage of tumors (Supplementary Table 3).

### Global expression – score

Global expression showed a wide variability among tumors. Our results showed significant differences in the score of some cytokines according to the different clinicopathological characteristics from patients and tumors (Table 1). Scores of IL-1β and IL-17 were positively associated with histological grade (SBR); whereas, node-negative tumors showed a higher global expression of IL-6, and HER-2-positive tumors showed a higher global expression of IFNβ but a lower global expression of NFκB.

The possible association between cytokines expression and prognosis in all patients included in the present study, was investigated. Figure 2 shows the association between the score values of each cytokine, dichotomized at the median, and both relapse-free survival (RFS) and overall survival (OS). This statistical analysis showed that a high score of IL-6 was significantly associated with both longer RFS (p=0.003) and OS (p=0.004) (Figure 2C-D).

**Cell type expression**

As it shown in the double-staining of IL-1β (as a representative example) with specific markers for cancer cells, CAFs and MICs (Figure 1B), these cells can express or not the studied factors. In addition, the morphological description done previously for each cell type corresponds to each one, as shown by the specific markers.

In positive cases, we found at least 60% of positives MICs or CAFs in each evaluated field.

The analysis of cytokines expression by each cell type according to the different clinicopathological characteristics from patients and tumors showed that node-negative tumors had a significant high percentage of cases with CAFs positives for IL-1β (number positive cases (percentage): 45(58.4%); p=0.029) or for IL-17 (35 (18.3%); p=0.006) than node-positive tumors. However, we found not other significant associations between clinicopathological characteristics and cell type expression (data not shown).

We also analyzed the relationship between cytokines expression by each cell type and prognosis. We found that IL-1β expression by CAFs or MICs was significantly associated with both longer RFS (p=0.002 for CAFs and p=0.007 for MICs) and OS (p=0.007 for CAFs and p=0.012 for MICs) (Figure 3A-D). By contrary, IL-10 expression by CAFs or MICs was significantly associated with both shortened RFS (p<0.001) and OS (p<0.001) (Figure 3E-H).

The other cytokines expression (IL-6, IL-17, IFNβ and NFkB) by CAFs or MICs did not reach a significant association with prognosis (data not shown).

**Combinations of cytokines expression by stromal cells**

In order to assess the prognostic potential of the stroma phenotype , different combinations of cytokines expression by CAFs and MICs were also evaluated. In this sense, our results indicate important associations (Figure 4). The combination of the expression of IL-1β and the non-expression of IL-10 by MICs was associated with a lower probability of RFS and OS, whereas the combination of the non-expression of IL-1β and the expression of IL-10 by MICs was associated with the poorest prognosis, for both RFS ($p<0.001$) and OS ($p=0.001$) (Figure 4 A-B). A similar and also significant association was found when we evaluate the combination of IL-1β and IL-10 expression by CAFs (Figure 4 C-D). There are significant correlation between the expression of IL-1β and IL-10 by CAFs and MICs (rsub: 0.728, $p<0.0001$). In addition, the combination of IL-1β, IL-6 and IL-10 expression by MICs also reached an important association with prognosis (Figure 4E-F), so that the group with non-expression of IL-1 β and IL-6 together with expression of IL-10 had the highest probability of distant metastasis development ($p<0.001$) and a shortened OS ($p=0.001$). Any other possible combinations achieved not significance to predict the prognosis (data not shown).

**Combinations of cytokines expression by stromal cells in a subgroup of tumors (MMP11-negative by MICs)**

Considering previous data of our group indicating that MMP-11 expression by MICs is a potent prognostic indicator (8, 11-12, 15), in the present study we also investigated the possible contribution of the analyzed cytokines expression to improve this prognostic value. In accordance with previous reports, tumors with MMP11-positive MICs had a worse

prognosis (Figure 5A-B) for both RFS ($p<0.001$) and OS ($p<0.001$). Therefore, we analyzed the prognostic value of the cytokines expression in order to improve the prognostic evaluation for patients with tumors with MMP11-negative MICs. Thus, our results indicate that high score of IL-6 (Figure 5C-D) but also the expression of IL-1β by stromal cells, CAFs and MICs, (Figure 5E-H) were related to a longer relapse free-survival (RFS) and overall survival (OS). Moreover, we analyzed the combination of the expression of IL-1β, IL-6 and IL-10 by MICs and we found a group of tumors characterized by the non-expression of IL-1β and IL-6 together with the expression of IL-10 (IL-1β - / IL-6 - / IL-10 +), which has the worst prognosis for both RFS ($p<0.001$) and OS ($p=0.002$) (Figure 5I-J).

**Multivariate analysis**

Multivariate analysis according to Cox's model, in the overall population of patients, demonstrated that tumor stage was significant and independently associated with RFS (tumor stage II: (relative risk (RR) (confidence interval (CI)=0.46 (0.41–0.57); tumor stage III: 0.61 (0.48–0.78); $p=0.004$). Moreover, this same analysis also demonstrated that the combination of the expression of IL-1β, IL-6 and IL-10 by MICs was significant and independently associated with distant relapse-free survival (8.257 (1.65-41.25); $p=0.010$).

## DISCUSSION

Cytokines are low-molecular-weight proteins that mediate cell-to-cell communication. Immune and stromal cells, such as fibroblasts and endothelial cells, synthesize cytokines and regulate through them several processes as proliferation, cell survival, differentiation, immune cell activation, cell migration, and death. Our results demonstrate that cytokines are express by key cell types from breast carcinomas (cancer cells, CAFs and MICs). Despite the fact that cytokines were mainly expressed by cancer cells, we found variability in their expression by CAFs and MICs among tumors, which seems to have clinical significance. Therefore, our findings also support the co-dominant role of stroma in tumor progression.

The cytokines studied were selected based on own previous reports (11-12), where we found that IL-1β, IL-6, IL-17, IFNβ and NFκB, were expressed at high levels in tumors with MMP-11-positive MICs, which are pro-metastatic tumors, as demonstrated in several studies (7-8, 10, 15). We also considered of interest to analyze the expression of IL-10 in the present study, because it is an important anti-inflammatory cytokine that contributes to tumor immune evasion (For review see refs. (16-18)). Almost all immune cells, including T cells, B cells, monocytes, macrophages, mast cells, granulocytes and dendritic cells, produce IL-10 (19). Tumor cells can also secrete IL-10, as can tumor-infiltrating macrophages (20-21). Due to its immunosuppressive effect on dendritic cells and macrophages, IL-10 can dampen antigen presentation, cell maturation, and differentiation, allowing tumor cells to evade immune surveillance mechanisms (22).

The finding of the present work about high cytokine expressions by cancer cells supports the recognized fact that that cancer cells secrete cytokines that can actas autocrine factors which

contribute to their malignant phenotype. In fact, cytokines beside their central role in inflammation, have also been recognized as powerful players in tumor progression via many pathways: including the generation of free radicals that can damage DNA, potentially causing mutations that lead to tumor initiation, stimulating cell proliferation and reducing apoptosis, stimulating epithelial-mesenchymal transition and angiogenesis, or allowing tumor cells evasion of immune surveillance. In accordance with these concepts, our data showed some significant associations, like positive relationships of global expression of IL-1β and IL-17 with moderately and more undifferentiated tumors, respectively, which was previously reported for IL-17 (23). Although, previous reports shown several positive associations between high levels of cytokines and tumor aggressiveness, most of them were based on serum levels and few of them evaluated the impact on RFS and OS (24-26). On the other hand, cytokines can modulate an anti-tumoral response, which seems conditional on the balance of pro- and anti-inflammatory cytokines, their relative concentrations, cytokine receptors expression content, and the activation state of surrounding cells (27), and the stage of tumor development (18). In this sense and how our results indicate, it is relevant to consider the cell type expressing cytokines in the context of the tumor environment. We found that IL-1β expression by CAFs or MICs was significantly associated with longer RFS and OS in our studied population of patients with breast carcinomas. By contrary, IL-10 expression by MICs or CAFs was significantly associated with a poor prognosis. These data seem to reflect the complexity of the tumor stroma. But, at the same time, they led us to identify a phenotype of stromal cells in which interleukins may have partial antitumor effect through promoting immune response in the tumor, which is defined as tumor immunosurveillance. Thus, these findings are in accordance with some studies suggesting that interleukins can inhibit tumor growth (28-29) and can be correlated with good prognosis (30-31). Nevertheless, it seems to depend on the cell type which expressed them, such as our

results suggest. With regard to CAFs, it is known that they are capable of evoking a pro-inflammatory response, since after activation they initiate the secretion of IL-1β, IL-6, IL-8, stromal cell derived factor-1 (SDF-1) and NFκB, which may induce inflammation by recruiting cell components of the immune system (32-33).

It is also remarkable our finding indicating that the combination of the non-expression of both IL-1β and IL-6 and the expression of IL-10, by MICs, was associated with the worst prognosis. These findings indicate that the combination of the expression of cytokines may led us to better identify phenotypes of stromal cells with different prognostic signification in breast cancer. Indeed, these results showed that the non-expression of pro-inflammatory cytokines (as IL-1β and IL-6), together with the expression of an anti-inflammatory cytokine (as IL-10) could contribute to tumor immune escape.

In the present study, we also investigated the possible contribution of these cytokines to improve the prognostic value of MMP-11 expression by MICs. As our previous reports indicate (8, 11-12, 15), tumors with MMP11-positive MICs had a worse prognosis, so that it is interesting to improve the prognostic evaluation in patients with tumors having MMP11-negative MICs. In this last patients' subgroup, our results indicate that IL-6 global expression, or IL-1β expression by CAFs or MICs, or the combination of IL-1β, IL-6 and IL-10 by MICs discriminate patients who differ significantly in their prognosis. Therefore, our data contribute to a more precise characterization of the cellular component in breast cancer stroma, in order to improve their prognostic value and their consideration to design further therapies. With regard to this last aspect, there are evidences indicating that tumor microenvironment is a fertile ground for the development of novel therapies with the potential to augment existing treatment and prevention options. Also, several clinical trials have been implemented in order

to evaluate inhibitors of cytokines receptors or neutralizing antibodies that prevent the sustained exposure to these inflammatory mediators that promote tumor progression (34-35). Nevertheless, such as our results suggest, it is very important to study the role of these mediators in different tumors or stages of development, since is essential for designing new personalized treatments using them as potential therapeutic targets.

In summary, our results indicate that some cytokines, such as the combination of IL-1β, IL-6 and IL-10 may serve as promising biomarker of MICs, with prognostic significance and potential clinical application prospect. Further studies, considering the morphological location of these expressions, may contribute to a better biological characterization of the tumor microenvironment from breast cancer.

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**FIGURE LEGENDS:**

**FIGURE 1: A)** Representative pictures of the immunohistochemical staining for the different cytokines analyzed in breast cancer samples (200X). (**Control (-)**) shows a representative picture of negative immunostaining without primary antibody (**a**) IL-1β, (**b**) IL-6, (**c**) IL-10, (**d**) IL-17, (**e**) IFNβ and (**f**) NFκB. Scale bar = 100 µm. **B)**Representative pictures of the double immunohistochemical staining for IL-1β and the different markers specific to each cell type (200X). Cytokeratin AE1/AE3 staining (red) specific for cancer cells alone (**a**) or in combination with IL-1β (brown) (**d**), α-SMA staining (red) specific for CAFs alone (**b**) or in combination with IL-1β (brown) (**e**), and CD45 staining (red) specific for MICs alone (**c**) or in combination with IL-1β (brown) (**f**). The cell type of interest was indicated by arrows in each staining. Scale bar = 100 µm.

**FIGURE 2:** Prognostic significance of global expression of each cytokine studied in mammary carcinomas. Kaplan–Meier survival curves for relapse-free survival (RFS) and overall survival (OS), as a function of the score of each cytokine, dichotomized taking the median score as the cut-off point. (**A**) RFS for IL-1β, (**B**) OS for IL-1β, (**C**) RFS for IL-6, (**D**) OS for IL-6, (**E**) RFS for IL-10, (**F**) OS for IL-10, (**G**) RFS for IL-17, (**H**) OS for IL-17, (**I**) RFS for IFNβ, (**J**) OS for IFNβ, (**K**) RFS for NFκB, and (**L**) OS for NFκB.

**FIGURE 3:** Prognostic significance of stromal expression of each cytokine studied in mammary carcinomas. Kaplan–Meier survival curves for relapse-free survival and overall survival, as a function of the expression of IL-1β by CAFs (**A**-**B**) or by MICs (**C**-**D**), or as a function of the expression of IL-10 by CAFs (**E**-**F**) or by MICs (**G**-**H**).

**FIGURE 4:** Survival analysis of breast carcinoma patients stratified according to the combination of the expression of different cytokines. Kaplan–Meier survival curves for relapse-free survival and overall survival, as a function of the combination of the expression of IL-1β and IL-10 by MICs (**A**-**B**) or by CAFs (**C**-**D**), or as a function of the combination of the expression of IL-1β, IL-6 and IL-10 by MICs (**E**-**F**).

**FIGURE 5:** Prognostic significance of the expression of different cytokines depending on the MMP-11 expression by MICs in breast carcinoma patients. Kaplan–Meier survival curves for relapse-free survival (RFS) and overall survival (OS), as a function of the expression of MMP-11 by MICs (**A**-**B**). RFS and OS curves in the population of tumors MMP-11 negative by MICs, as a function of the score of IL-6, dichotomized taking the median score as the cut-off point (**C**-**D**), as a function of the expression of IL-1β by CAFs (**E**-**F**) or by MICs (**G**-**H**), or as a function of the combination of the expression of IL-1β, IL-6 and IL-10 by MICs (**I**-**J**).

**Table 1:** Relationship between cytokine immunostaining score values and clinicopathological characteristics in 108 patients with breast cancer.

| CHARACTERISTICS | IL-1β | IL-6 | IL-10 | IL-17 | IFN-β | NFκB |
|---|---|---|---|---|---|---|
| **Total cases** | 163.7 (0-281.0) | 63.6 (0-231.4) | 129.0 (0-262.5) | 159.5 (0-291.1) | 147.3 (56.4-266.8) | 147.9 (0-291.6) |
| **Age median (years)** | | | | | | |
| ≤53 | 166.8 (0-278.6) | 62.3 (0-231.4) | 123.4 (0-234.7) | 156.1(39.5-291.1) | 149.0(61.1-245.4) | 150.1 (0-291.6) |
| >53 | 161.4(54.8-281.0) | 68.2 (0-177.4) | 131.2 (0-262.5) | 161.8 (0-288.8) | 144.2(56.4-266.8) | 143.6 (0-266.7) |
| **Menopausal status** | | | | | | |
| Premenopausal | 166.8 (0-274.9) | 61.5 (0-231.4) | 126.0 (0-234.7) | 156.1(39.5-286.6) | 145.2(61.1-266.8) | 146.4 (0-240.7) |
| Postmenopausal | 163.7(54.8-281.0) | 68.2 (0-177.4) | 129.4 (0-262.5) | 161.5 (0-291.1) | 145.4(56.4-265.5) | 148.6 (0-291.6) |
| **Tumor size** | | | | | | |
| T1 | 163.1(65.9-273.7) | 65.0 (0-168.0) | 132.6 (0-234.7) | 155.9 (0-288.8) | 150.2(56.4-265.5) | 143.6 (0-266.7) |
| T2 | 164.2 (0-281.0) | 63.6 (0-231.4) | 125.8 (0-262.5) | 161.7(39.5-291.1) | 138.7(63.7-266.8) | 151.0 (0-291.6) |
| **Lymph node status** | | ***p*=0.018** | | | | |
| N- | 162.5(53.9-273.7) | **85.1 (0-177.4)** | 130.8 (0-262.5) | 161.1 (0-291.1) | 145.1(56.4-265.5) | 147.1 (0-291.6) |
| N+ | 166.8 (0-281.0) | **49.8 (0-231.4)** | 119.4 (0-248.9) | 157.3(39.5-268.5) | 149.8(63.7-266.8) | 148.3 (0-273.7) |
| **Tumor grade** | | | | | | |
| I | 176.3(68.5-273.7) | 87.9 (0-168.0) | 136.2 (0-234.7) | 154.0 (0-288.8) | 138.1(56.4-265.5) | 125.6(64.6-266.7) |
| II | 166.8 (0-275.0) | 72.6 (0-231.4) | 130.4 (0-262.5) | 162.3(67.8-291.1) | 143.9(63.7-266.8) | 148.6 (0-240.7) |
| III | 163.7(73.6-281.0) | 59.9 (15.6-154.0) | 127.2 (44.8-230.4) | 155.6(69.7-268.2) | 148.8(71.6-247.2) | 141.6(50.0-273.7) |
| **Histologic grade** | ***p*=0.032** | | | ***p*=0.010** | | |
| Well diff. | **148.4(53.9-275.0)** | 71.4 (0-177.4) | 103.3 (0-234.7) | **149.4 (0-288.8)** | 132.7(56.4-242.7) | 129.6 (0-223.8) |
| Mod diff. | **177.1 (0-281.0)** | 63.3 (0-159.0) | 129.1 (0-248.9) | **155.6(39.5-281.3)** | 153.5(65.6-266.8) | 147.9 (0-291.6) |
| Poorly diff. | **166.3 (0-278.9)** | 63.6 (3.1-231.4) | 131.2 (42.7-262.5) | **172.6(67.8-291.1)** | 145.3(63.7-265.5) | 152.8 (0-273.7) |
| **Estrogen receptors** | | | | | | |
| Negative | 166.8(54.8-275.1) | 65.8 (3.1-152.2) | 126.0 (38.9-227.1) | 158.7(65.4-291.1) | 138.4(70.4-265.5) | 144.6(0-291.6) |
| Positive | 162.6 (0-281.0) | 70.0 (0-231.4) | 130.6 (0-262.5) | 160.3 (0-288.8) | 149.4(56.4-266.8) | 149.7 (0-273.7) |
| **Progesterone receptors** | | | | | | |
| Negative | 166.8 (0-281.0) | 65.8 (3.1-168.0) | 129.2 (38.9-234.7) | 159.0(39.5-291.1) | 142.9(61.1-265.5) | 148.6 (0-291.6) |
| Positive | 162.6(53.9-278.9) | 69.4 (0-231.4) | 125.5 (0-262.5) | 159.5 (0-288.8) | 149.4(56.4-266.8) | 147.2 (0-273.7) |
| **HER2 status** | | | | | ***p*=0.023** | ***p*=0.021** |
| Negative | 163.4 (0-278.9) | 63.4 (0-231.4) | 124.8 (0-262.5) | 156.3 (0-291.1) | **141.0(56.4-266.8)** | **242.7 (0-291.6)** |
| Positive | 175.2(54.8-281.0) | 65.1 (15.6-165.2) | 131.0 (44.7-183.96) | 177.6(89.3-286.6) | **152.8(72.3-156.1)** | **159.1 (0-265.8)** |
| **Basal group** | | | | | | |
| No basal-like | 161.7 (0-281.0) | 67.5 (0-231.4) | 130.8 (0-262.5) | 160.9 (0-288.8) | 149.0 (56.4-266.8) | 149.7 (0-273.7) |
| Basal-like | 168.5 (74.1-275.0) | 65.8 (3.1-152.2) | 116.1 (38.9-227.1) | 157.7 (65.4-291.1) | 140.2 (70.4-265.5) | 143.6 (0-291.6) |
| **Adjuvant radiotherapy** | | | | | ***p*=0.046** | |
| No | 166.0(53.9-275.0) | 75.9 (0-231.4) | 127.4 (0-262.5) | 161.5 (0-291.1) | **140.5(61.1-256.1)** | 149.3 (0-291.6) |
| Yes | 159.1 (0-281.0) | 55.0 (0-159.0) | 132.6 (27.4-230.4) | 157.7(39.5-281.0) | **150.6(56.4-266.8)** | 146.4 (0-273.7) |
| **Adjuvant systemic therapy** | | | | | ***p*=0.025** | |
| TMX | 165.2(68.5-269.9) | 85.9 (0-231.4) | 141.9 (0-262.5) | 162.0 (0-288.8) | **150.2(63.7-256.1)** | 141.2(13.8-245.6) |
| CMT | 151.4 (0-273.7) | 73.1 (3.1-177.4) | 120.8 (27.4-227.1) | 157.1(65.4-291.1) | **144.8(56.4-265.5)** | 145.6 (0-291.6) |
| CMT+TMX | 169.6 (0-281.0) | 58.0 (0-175.5) | 128.9 (0-230.4) | 158.7(39.5-286.6) | **147.5(63.7-266.8)** | 151.2(50.0-273.7) |
| No treatment | 202.5 (60.6-270.4) | 38.6 (0-155.7) | 141.5 (77.1-180.9) | 154.0 (75.5-243.3) | **71.9 (61.1-242.7)** | 154.9 (64.9-215.1) |

Data are expressed as median (range). Statistic tests applied: Mann whitney or Kruskal-Wallis. TMX: Tamoxifen; CMT: chemotherapy.

**Figure 1**

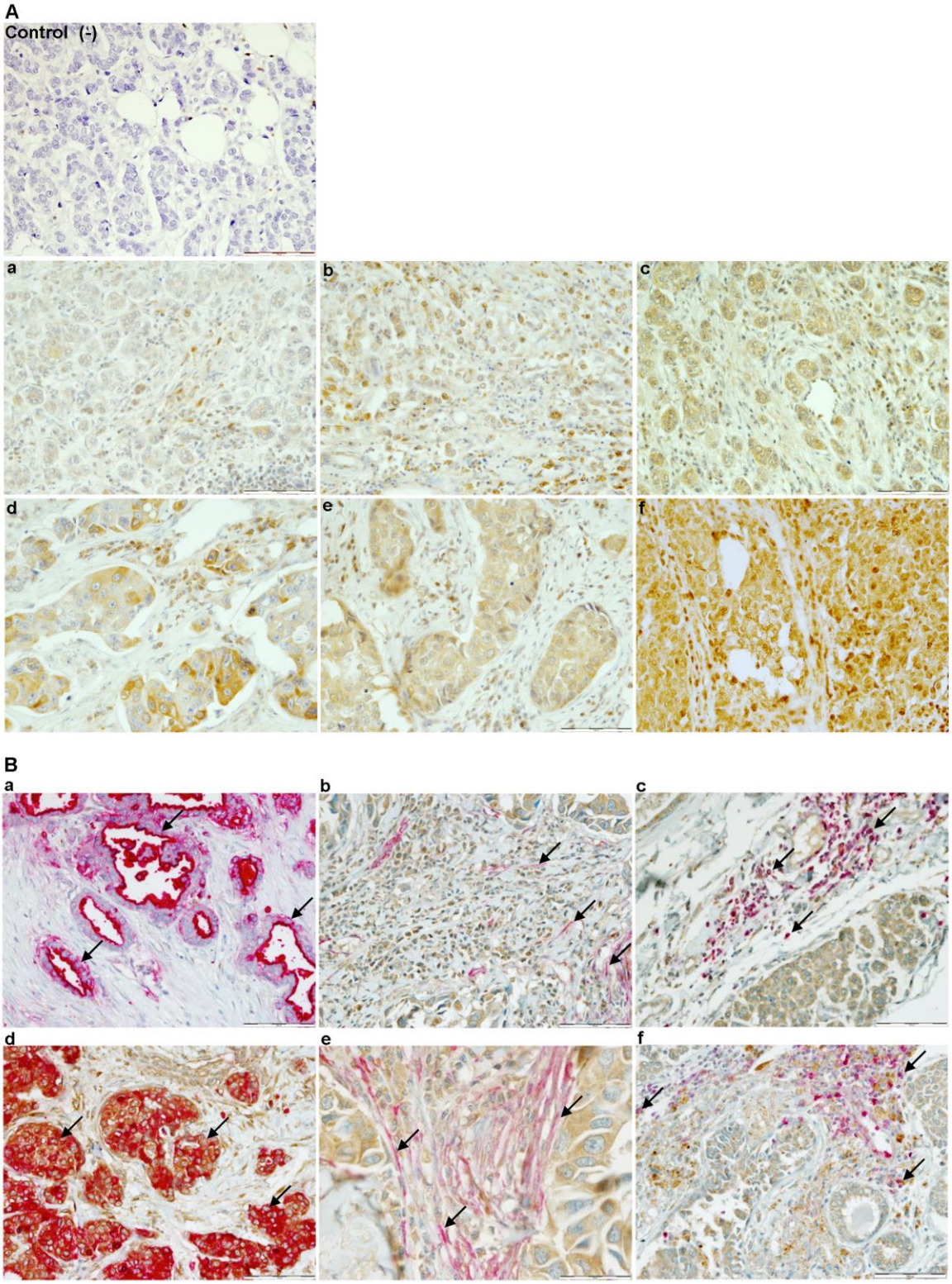

**Figure 2**

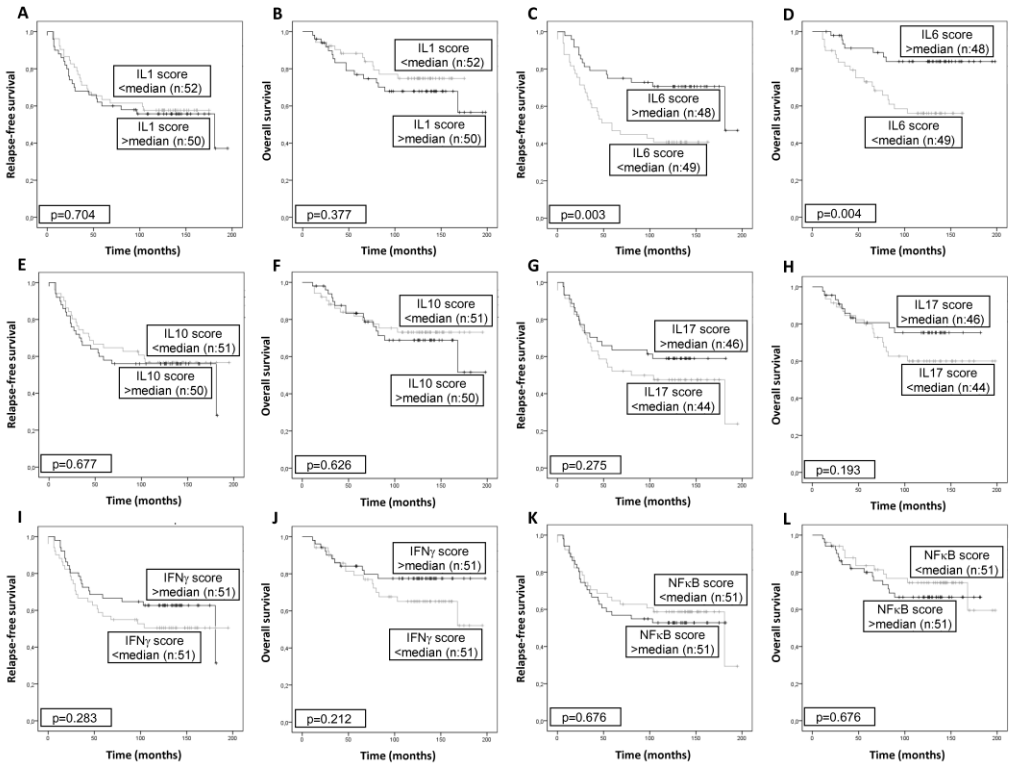

**Figure 3**

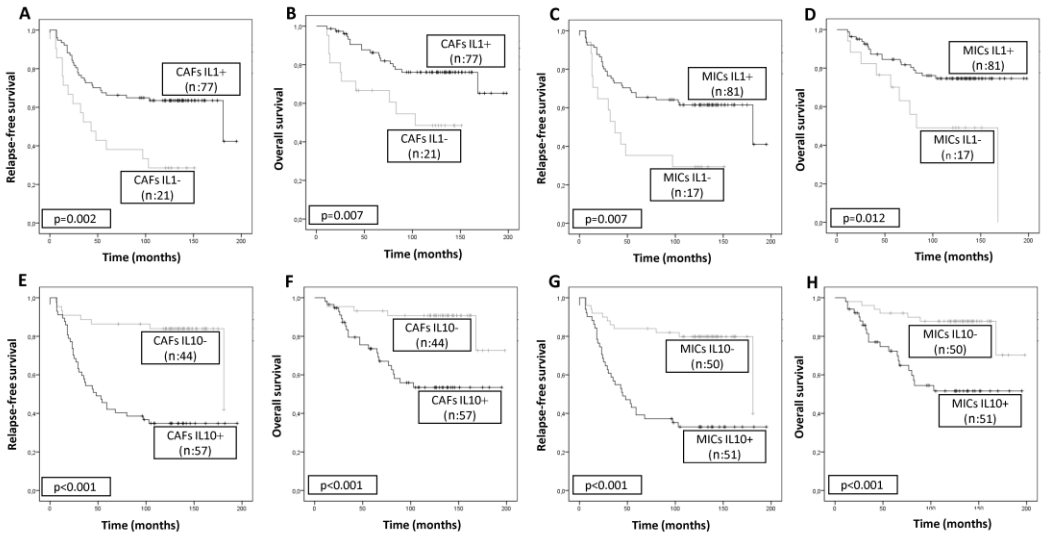

**Figure 4**

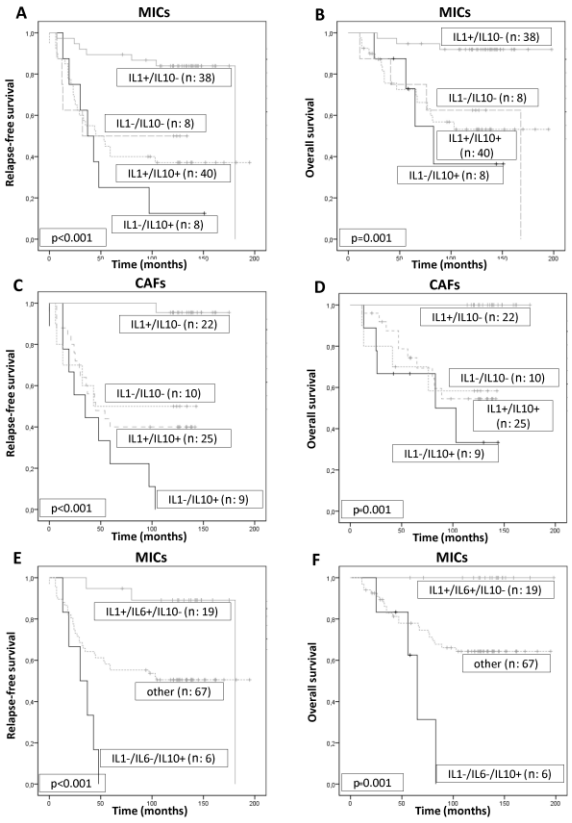

**Figure 5**